Evaluating the Anti-Obesity Effects of Chitosan in High-Fat Diet-Induced Obesity in Rat Model

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Abstract

Background: Chitosan oligosaccharide (COS) exhibits hypolipidemic and anti-obesity properties. Nevertheless, it remains uncertain whether its efficiency exhibits sex-specific variations. Therefore, this study seeks to fill this gap, systematically evaluating the impact of chitosan oligosaccharides across genders under obesogenic conditions. Material and methods: 36 rats: 18 male, 18 female; split into 6 groups, each with equal male/female. G1 had a standard diet; G2 had a high-fat diet for 4 weeks, then standard for another 4 weeks; G3 had a high-fat diet for 4 weeks, then 500 mg/kg chitosan + standard diet for another 4 weeks. At the study's end, rats were euthanized, blood was collected for biochemical analysis, and liver tissues were excised for histological assessment. Results: Male rats on a high-fat diet displayed significantly elevated body weight, triglycerides, cholesterol, lactate dehydrogenase, ALT, AST, and total protein levels, coupled with noticeable liver damage compared to female rats. However, when treated with COS, male and female rats exhibited reduced weight, lowered triglycerides, cholesterol, lactate dehydrogenase, ALT, and AST levels, and improved liver function and structure. Interestingly, these therapeutic effects were more evident in female and male rats.

Conclusion: The study found that COS administration to rats, especially females, reduced weight, and improved liver health, suggesting its potential gender-specific effects on a high-fat diet.

Keywords: Chitosan oligosaccharides, HFD, Gender, Liver
Introduction
The escalating global prevalence of obesity presents a significant public health challenge, driven primarily by lifestyle factors such as sedentary habits and high-fat diets affecting both adults and children [1-4]. The World Health Organization (WHO) predicts an alarming increase in obesity-associated health complications, anticipating more than 167 million individuals to suffer from these issues by 2025 [5]. This epidemic carries extensive health implications, including metabolic syndrome, hypertension, cardiovascular diseases, diabetes, and nonalcoholic fatty liver disease (NAFLD) [1, 2, 5]. Obesity is associated with systemic oxidative stress, stemming from the peroxisomal and mitochondrial oxidation of fatty acids in adipose tissues, ultimately resulting in liver inflammation, damage, and potential failure [1]. Globally, NAFLD affects 25% of the population, progressing from fatty liver to potentially severe conditions marked by the buildup of triglycerides in the liver, often associated with metabolic risk factors [6]. Anti-inflammatory or antioxidant food supplements may help prevent and treat NAFLD that results from a high-fat feeding regimen [7]. Obesity treatment involves diet, exercise, medications, and surgery. Current medications have drawbacks like toxicity and rebounding [8]. Considering obesity's multifaceted nature, researchers are exploring adjunctive therapies beyond conventional methods. Chitosan, derived from chitin in various organisms, has gained attention due to its potential lipid-binding attributes and safety profile [9, 10]. Particularly, chitosan oligosaccharide, with its low molecular weight and water solubility, holds promise in nutritional research for reducing lipid levels and offering diverse health benefits [11-13]. Those health benefits include anti-diabetic, anti-cancer, anti-inflammatory, hypolipidemic, antioxidant, and immune stimulatory mechanisms [4, 6, 13].

Gender-specific responses to dietary interventions are crucial; sex differences influence obesity rates due to hormonal and body composition variations [14]. Notably, investigations on chitosan's impact have primarily focused on males, prompting the need to explore its efficacy in obese female models [13]. High-fat diets serve as a model for studying obesity-related traits; however, differences between sexes and rodent species under these diets remain poorly understood [15]. This article examines weight, lipids, and liver health responses to assess chitosan's gender-specific effects in obese rat models. Insights may guide personalized dietary interventions for improved health outcomes.

Material and methods
Chemical and diets
Five grams of chitosan (Alpha Chemika, Mumbai, India) were dissolved in a 5% acetic acid solution (Thermo Fischer Scientific) at 55°C for 15 minutes. High-Fat Diet Composition: Comprised: Normal chew (54%), sucrose (15%), lard (15%), egg yolk powder (5%), milk powder (4%), peanut (3%), salt (2%), sesame oil (1%), dicalcium phosphate (0.6%), and mountain flour (0.4%).

Ethical Approval and Experimental Design
The Ethical Committee for Experimental Use of Animals at Damanhur University, Egypt (No: DMU-SCI-CSRE-23-09-01) ratified the rules for laboratory animal care and use. Pathogen-free albino rats of both sexes were obtained from the animal shelter associated with the Faculty of Science at Damanhur University. Rats were individually housed under specified conditions: a 22 ± 2°C temperature range, a 12-hour light/dark cycle, and a 30–50% humidity range. Male and female rats were assigned to three groups of equal numbers:

1. Control Group (G1): Rats were fed a regular rodent chew for 8 weeks.
High-Fat Diet (HFD) Group (G2): Rats were fed the HFD for 4 weeks, then switched to be fed regular chew for another 4 weeks.

HFD + COS Group: Rats were fed the HFD for 4 weeks and then administered a daily dose of 500mg/kg COS [12] alongside regular chew for an additional 4 weeks.

Sample Collection and Processing:
Following overnight fasting, rats were anesthetized with 1% sodium pentobarbital, sacrificed, and blood was collected from the abdominal aorta. Serum was obtained by centrifugation (3500 r·min⁻¹ for 15 minutes) and kept at −80°C. Liver tissues were excised, rinsed in normal saline, and fixed in 10% formalin.

Biochemical Parameter Analysis:
Serum samples were analyzed for triglycerides, total cholesterol, VLDL-C, lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total protein levels utilizing commercial kits following standard protocols.

Biochemical Parameter Analysis

Determination of Triglyceride and total cholesterol
The Triglyceride and total cholesterol levels in serum were measured using commercial kits from Biodiagnostic, Egypt (Cat. No. CH 12 20 and Cat. No. TR 20), performed as per manufacturers' instructions based on Richmond [16] and Fassati, Prencipe [17], respectively methods. The very-low-density lipoprotein levels were estimated using the formula VLDL-C = Triglycerides / 5, as reported by DeLong et al. [18].

Determination of Lactate Dehydrogenase (LDH)
The levels of lactate dehydrogenase (LDH) were estimated using the colorimetric kits (Cat. No. E-BC-K046-S, Elabscience Biotechnology Inc., USA) based on the protocols described by Holbrook et al. [19].

Determination of liver function enzymes and total protein
Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total protein levels were assessed utilizing the enzymatic techniques established by Reitman, Frankel [20] and Henry [21]. Diamond Diagnostics kits (CAT. NO. AL 10 31 for ALT, CAT. NO. AS 10 61 for AST, and CAT. NO. TP 20 20 for total protein) sourced from Egypt were employed for these measurements.

Histopathological investigation
Liver tissues fixed in formalin for 24 hrs were dehydrated in gradually increased concentrations of ethanol alcohol, cleared twice in xylene, and embedded in paraffin, as outlined by Suvarna et al. [22]. Those embedded samples were sectioned and stained with hematoxylin and eosin (H&E). An Olympus CX40 microscope was used to examine the sections.

Statistical Analysis
Data is displayed as means ± SD, and statistical significance was calculated using GraphPad software's one-way ANOVA and Tukey's post hoc test.

Results
Body Weight Change
Figure 1 illustrates the alterations in rat body weight over the 8-week trial. Initially, all groups exhibited similar body weights; however, by the second week, male and female rats fed HFD (G2&G3) began displaying significantly higher body weights than control rats fed a regular diet (G1). Ultimately, at the trial's conclusion, the high-fat diet groups (HFD and HFD+COS) in both sexes exhibited significantly higher body weights compared to the control groups. Remarkably, the administration of COS (G3) significantly lowered the body weight of G3 rats compared to the untreated HFD-fed groups (G2) (Figure 1B). HFD+COS–treated female group
showed lower body weight than HFD+COS–treated male group.

Serum levels of lipid and lactate dehydrogenase

Figure 2 presents data on lipid and lactate dehydrogenase levels. Untreated HFD-fed rats (G2) showed significant elevations in triglycerides, VLDL-C, total cholesterol, and lactate dehydrogenase compared to controls (G1). However, HFD+COS-treated rats (G3) exhibited significant reductions in these levels compared to untreated HFD-fed rats (G2). Interestingly, HFD+COS-treated rats (G3) displayed slight increases in triglycerides, VLDL-C, and total cholesterol, with a significant rise in lactate dehydrogenase compared to controls (G1).

Liver function markers

Figure 3 displays statistical variations in ALT, AST, and total protein. Untreated HFD-fed rats (G2) exhibited significantly higher ALT, AST, and total protein levels than control groups (G1). However, COS administration (G3) led to a significant decline in ALT and AST levels when compared to the untreated HFD-fed groups (G2) (Figure 3A-B). Specifically, COS+HFD-treated male rats significantly decreased total protein levels compared to untreated HFD-fed male rats (Figure 3C). Contrastingly, HFD+COS-treated male rats demonstrated significantly higher ALT levels than control males, alongside increased AST and total protein levels. Additionally, HFD+COS-treated female rats displayed slight increases in ALT, AST, and total protein compared to control female rats, although these changes were not statistically significant.

Figure 1. Body weight changes in various experimental groups. A. Graphical depiction of body weight changes in various experimental groups across different weeks of the experiment. B. Histogram showing alterations in final body weight among different experimental groups. Data presented as means ± SD. * denotes significant difference compared to control groups (*p < 0.05, **p < 0.01, *** p < 0.001), while # represents significant difference compared to HFD-FED-treated groups (#p < 0.01, ### p < 0.001).
Figure 2. The effect of chitosan treatment on the levels of (A) triglycerides, (B) very low-density lipoprotein cholesterol (VLDL-C), (C) cholesterol, and (D) lactate dehydrogenase (LDH) in different experimental groups. Data presented as means ± SD. * denotes significant difference compared to control groups (*p < 0.05, **p < 0.01, *** p < 0.001), while # represents significant difference compared to HFD-FED-treated groups (#p < 0.05, ##p < 0.01, ### p < 0.001).
Figure 3. The effect of chitosan treatment on the levels of (A) ALT, (B) AST, and (C) Total protein in the serum of different experimental groups. Data presented as means ± SD. * denotes significant difference compared to control groups (***p < 0.01, **p < 0.001), while # represents significant difference compared to HFD-FED-treated groups (##p < 0.01, ###p < 0.001).
Liver Histology:

Control Group:
Liver sections from male and female rats in the control group (G1) displayed normal liver morphology. Hepatocytes formed cord-like structures, separated by blood sinusoids extending from the central vein to the portal areas (Figure 4A-B). These sinusoids were lined with endothelial cells and Kupffer cells. Hepatocytes showed polygonal shapes, large basophilic nuclei with distinct nucleoli, and homogeneous cytoplasm (Figure 4C-D). Typical hepatic triad elements were present in the portal regions, including portal vein branches, hepatic artery branches, bile ductules, and connective tissue fibers (Figure 4E-F).

Untreated HFD-fed Group:
Livers of untreated HFD-fed male and female rats (G2) exhibited significant liver damage, with distorted hepatic architecture and unclear hepatic cords and blood sinusoids, mainly in the periportal area (Figure 5A-D). Hepatocytes displayed ballooning, numerous cytoplasmic vacuoles, and hyperchromatic or disintegrated nuclei, indicating necrosis (Figure 5C-D). Moreover, hepatic Langerhans cell histiocytosis lesions with severe leucocyte infiltration were observed (Figure 5 E-F). Portal vein dilation, congestion, wall hyperplasia, and increased bile ductule proliferation were also observed (Figure 5E-F). These pathological changes were more pronounced in the livers of HFD-fed male rats than in females.

HFD-fed Group Treated with Chitosan (HFD+COS):
Liver sections from HFD+COS-treated groups (G3) showed restored hepatic lobule structure, marked by organized hepatocytes forming distinct cords separated by blood sinusoids (Figure 6A-B). Hepatocytes displayed slight cytoplasmic vacuolization but maintained normal nuclei (Figure 6C-D). Typical portal triad elements were also observed (Figure 6E-F). Notably, COS effectively reversed the HFD-induced effects, particularly in HFD-fed female treated rats, displaying almost typical liver architecture with minimal cytoplasmic vacuolization compared to HFD+COS treated males.
Figure 4. Liver sections of male and female control rats. A-B: showing central vein (CV) surrounded by hepatic cords separated by blood sinusoids (BS). C-D: high magnification showing normal hepatocytes with prominent nuclei (N), kupffer cells (KC), and endothelial cells (E) lining the blood sinusoids. E-F: showing portal vein (PV), bile ductule surrounded by small connective tissue. H&E
Figure 5. Liver sections of untreated obese male and female rats. A-B: Showing distorted hepatic structure with a dilated central vein (Yellow arrows) surrounded by leucocytic infiltration (curled blue arrows). C-D: high magnification showing hypertrophied hepatocytes with small dark nuclei and vacuolated cytoplasm (Astrics), hepatocytes necrosis (black circles), and hyperchromatic nuclei (Black squares). E-F: showing hepatic Langerhans cell histiocytosis (LCH) and leucocyte infiltration (black curled arrow). G-H: showing congested portal vein (blue arrows) with intimal hyperplasia (head arrow), bile ductules proliferation (black arrow), and leucocyte infiltration (black curled arrows). H&E
Figure 6. Liver sections of obese male and female rats treated with chitosan. A-B: Showing normal hepatic cord arrangement raised from the central vein (CV) and separated by blood sinusoids (BS). C-D: High magnification showing hepatocytes with regular size nuclei (N) and mild cytoplasmic vacuolization, sinusoidal lining Kupffer cells (KC), and endothelial cells (E). E-F: showing leucocyte infiltration (curled arrow), portal vein (PV), hepatic artery (HA) and bile ductules (BD). H&E

Discussion.
The research focused on the impact of a high-fat diet on lipid metabolism, liver function in both male and female rats, and the potential effectiveness of chitosan oligosaccharide (COS) in managing associated complications. This diet elevates the risk of obesity-related diseases, such as cardiovascular issues, diabetes, and impaired liver and pancreas, emphasizing the necessity for interventions that mitigate side effects [3, 8, 23]. Chitosan, recognized for its potential in modulating lipid metabolism and weight gain inhibition [1], was explored in this context.
Notably, gender-based variations influence obesity-related disease risks, evident in male rats displaying higher weight gain on a high-fat diet compared to females [14]. The study revealed increased weight gain in male rats on a high-fat diet compared to their female counterparts, aligning with prior findings suggesting males exhibit greater caloric intake and
weight gain in similar dietary conditions [14]. An excessive sucrose diet caused obesity in both male and female rats, but male rats were heavier than female rats, suggesting a protective role of estrogens against obesity [24]. Chitosan's mechanisms in preventing weight gain involve accelerating lipid breakdown and forming emulsified micelles with cholesterol and lipids, hindering their absorption and promoting excretion [4, 13, 25]. COS administration to HFD-fed rats significantly reversed the significant weight gain observed compared to untreated HFD-fed rats, aligning with previous studies indicating its potential to regulate body weight and appetite in HFD-induced obesity [2, 13]. These results underscore the potential of chitosan oligosaccharides in managing weight and appetite in obesity.

Serum lipid profile aberrations are vital indicators for obesity-related complications [26]. An increase in LDH levels, seen in conditions such as heart attacks, liver disease, muscle injury, anemia, and some cancers, is indicative of tissue damage or cellular breakdown [27]. The study highlighted hyperlipidemia in HFD-fed rats, indicated by elevated levels of serum triglycerides, VLDL-C, cholesterol, and lactate dehydrogenase. These results are consistent with Albrahim, and Alonazi [28], who reported significant elevations in triglycerides, cholesterol, and LDH levels in HFD-obese rats. The observed elevation in VLDL-C and cholesterol indicated a close association with nonalcoholic fatty liver disease [29].

Additionally, administration of an HFD to mice resulted in a significant elevation in triglycerides and cholesterol, marking the development of hyperlipidemia, a critical risk factor for cardiovascular diseases [26]. Furthermore, high blood triglyceride levels may also cause the release of pro-inflammatory cytokines, fibrinogen, and coagulation factors, which can lead to a thrombus inside the vascular lumen [30]. Increased LDH activity in untreated HFD-fed groups suggested cellular damage and oxidative stress, reinforcing the metabolic disruptions associated with high-fat diets [31]. Chitosan prevents weight gain by increasing lipid breakdown and acting as a carrier for cholesterol and triglycerides [1]. The study revealed significant reductions in triglycerides, VLDL-C, and total cholesterol levels due to COS administration, consistent with previous research [6, 8, 13]. Moreover, COS positively affects liver lipid metabolism, enhancing reverse cholesterol transport, reducing levels of triglycerides and LDL-C, and defending against oxidative stress [7, 32-34]. Ugbaja et al. [35] proposed that chitosan may offer a safe therapeutic alternative for managing cardiometabolic complications by reducing lipid accumulation, oxidative stress, and cardiac damage in HFD-fed rats. Studies indicate that COS administration significantly decreases LDH activity, potentially protecting against cellular damage and oxidative stress [27, 36].

The liver is critical in lipid synthesis and metabolic disruptions [8]. Hypertriglyceridemia is a significant feature in obesity-induced dyslipidemia, primarily due to increased free fatty acid influx into the liver, leading to elevated plasma cholesterol, ALT, and AST levels [23]. This study reported a significant increase in ALT, AST, and total proteins in untreated HFD-fed rats. Previous studies show that HFD administration in rodents caused liver damage, with elevated ALT and AST levels, highlighting the liver's vulnerability to lipid accumulation and functional alterations [26-28]. Chang et al. [37] reported that mice fed an HFD showed higher serum ALT and AST levels, indicating liver injury and hepatotoxicity linked to hyperlipidemia and fatty liver.

COS administration to HFD-fed rats significantly reduced ALT and AST levels, suggesting a curative effect against HFD-induced liver damage. Treatment
of rodents with COS reduces elevated levels of AST and ALT induced by the HFD regime, suggesting that COS may protect against liver damage induced by the HFD [6, 7, 34].

In the current study, untreated HFD-fed rats showed liver damage marked by hepatocyte cytoplasmic vacuolization and cellular necrosis with inflammatory cell infiltration in the portal area. Similarly, previous studies reported severe hepatocyte necrosis, periportal inflammation, and mild portal triad connective tissue increase in untreated HFD-fed rats [4, 28]. These data are inconsistent with Chinchu et al. [15], who reported that untreated HFD-fed rats have ballooning hepatocyte degeneration around the central vein and intracytoplasmic fat vacuoles with infiltration of inflammatory cells. Langerhans cell histiocytosis is characterized by cells similar to bone marrow-derived Langerhans cells infiltrated in various tissues and organs [38]. Nonalcoholic fatty liver disease originates from hyperlipidemia, leading to liver failure and Langerhans cell histiocytosis infiltration [39]. In the present study, hepatic Langerhans cell histiocytosis was also observed in liver sections of untreated HFD-fed rats. Fu et al. [40] documented that the excessive growth and buildup of Langerhans cells in the liver can lead to liver malfunction or the development of a mass lesion.

In the current study, COS administration to HFD-fed rats significantly improved liver structure by decreasing the hepatocytes' vacuolization, necrosis, and inflammatory cell infiltration. This data is inconsistent with Ugbaja et al. [4], who reported that chitosan improved the histology of the liver and reduced hyperlipidemia-induced oxidative stress in chitosan-HFD-fed rats. COS significantly improved the liver condition in HFD-fed rats by reducing cytoplasmic vacuolization, lipid content, and inflammation, thereby regulating pathways related to lipid accumulation [6]. Moreover, COS effectively reduced excessive fat accumulation, as evidenced by the decreased weight, and reversed HFD-induced fat vacuole accumulation in hepatocytes, highlighting its ameliorative effect on hepatic steatosis [34].

In conclusion, COS treatment demonstrated the ability to lower weight, ameliorate hepatic lipid accumulation, and reduce hepatic injury, thus reducing hepatic enzyme leakage and hepatic inflammation, ultimately alleviating hepatic injury in rats. In comparison to males, females are more likely to experience hypolipidemic and liver restoration effects from COS.

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